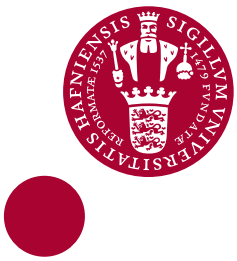

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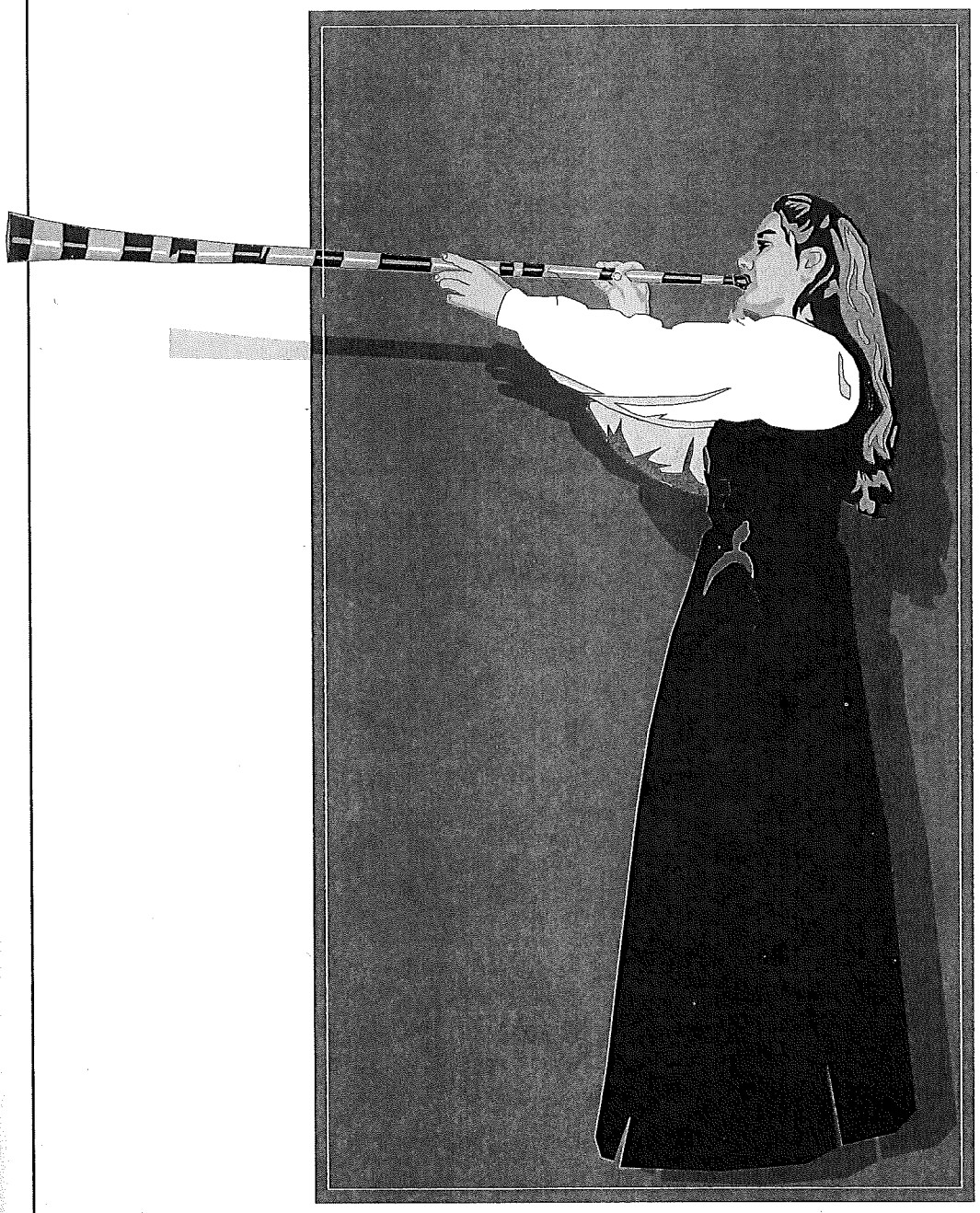

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Biocontrol of class IIa bacteriocin sensitive and resistant *Listeria monocytogenes* in sliced meat products

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Abstract

Controlled use of lactic acid bacteria has been envisioned as a possible way of reducing the load of chemical preservatives. We have investigated the applicational potential of a commonly occurring bacteriocin-producing lactic acid bacterium, *Leuconostoc carnosum* DMRICC 4010, for biocontrol of a cooked, sliced, modified atmosphere-packed meat product. In this product, *Listeria monocytogenes* grew from 10 CFU/g to a level of 10^5 CFU/g during four weeks storage at 5°C. By adding *Leuc. carnosum* at 10^7 CFU/g by a combination of sprinkling the surface of the unsliced product and spraying during slicing the initial level of *L. monocytogenes* was reduced. If the application was done only by spraying, or if an initial level of 10^4 CFU/g *Leuc. carnosum* was used, an inhibitive effect on growth was obtained, however no marked decrease in the initial level occurred. The biocontrolling culture could also inhibit growth of a bacteriocin-resistant mutant of *L. monocytogenes*. The culture addition did not affect the sensory quality of the product. In summary, we have developed a biocontrol procedure that can be used for large-scale operations. The work has resulted in a temporary approval for the use of *Leuc. carnosum* DMRICC 4010 for biopreservation of meat products.

Introduction

Vacuum or modified atmosphere-packed ready-to-eat meat may be stabilised to avoid growth of *L. monocytogenes* by using different chemical preservatives. Biocontrol has been suggested as an alternative to chemical preservation. Bacteriocin producing strains of *Leuc. carnosum* are frequently isolated from commercially vacuum-packed sliced meat products. We found these strains in approximately one third of the sliced meat products analysed for bacteriocin-producing lactic acid bacteria [1]. The strain chosen for further analysis, *Leuc. carnosum* DMRICC 4010, had a listericidal effect, and produces class IIa bacteriocins [1, 2].

Several problems can be anticipated in the practical application of biocontrolling cultures. We have investigated the efficiency of the biocontrol as a function of the initial level of the inhibitory culture, the method of dispersion on the product, and the bacteriocin sensitivity of the listerial strain. Additionally, we have tested the sensory quality of a biopreserved product.

Materials and Methods

Meat model

Pork saveloys were manufactured according to an industrial formulation comprising shoulder, jowls, soy isolate, potato starch, food grade sodium polyphosphates, sodium chloride, and sodium nitrite (60 ppm added). The chopped and ground ingredients were stuffed in casings and steam pasteurised at 80°C for 50 min, reaching a core temperature of 75°C. The products were cooled by water sprinkling and stored at 2°C. The final product had a pH of 6.2.

Bacterial strains and sample preparation

Lyophilised *Leuc. carnosum* DMRICC 4010 [1, 3], produced by Chr. Hansen A/S (Hørsholm, Denmark), was resuspended in 0.9 % NaCl at appropriate concentrations. Five isolates of *L. monocytogenes* were chill-propagated for 13 days at 5°C in BHI broth supplemented with 1.5 % NaCl, and mixed at equal volumes. The bacteriocin resistant *L. monocytogenes* strain was propagated in a similar way.

The saveloys were peeled and the surfaces were inoculated with *L. monocytogenes* (approx. 10 CFU/g). The inoculated saveloys were sliced in 0.2 cm slices on a SL 482 slicer (Dixie-Union Verpackungen GmbH, Germany). The *Leuc. carnosum* solution was added at approx. 10^4 or 10^7 CFU/g during slicing using a Disinfector 200® system (Clean Tech, Odense, Denmark) equipped with two nozzles, one facing the slicing direction, and the other perpendicular to the slicing direction [4]. Additionally, the surface of some saveloys were sprinkled with the *Leuc. carnosum* solution prior to slicing.

The inoculated slices were packed under modified atmosphere (20% CO_2 / 80% N_2) in vacuum bags of OTR = 0.45 $cm^3/m^2/atm/h$ (Danisco Flexible, Lyngby, Denmark). Packages were evacuated, back flushed, and heat-sealed (Multivac A300/16, Wolfertschwenden, Germany), resulting in a product/gas volume ratio of 1:4. The samples were stored at 5°C for four weeks.

Microbiological and sensory analyses

For samples containing less than 100 CFU/g, the 3 x 3 tube MPN-method in ½Fraser Broth was used for quantification of *L. monocytogenes*. For samples with more than 100 CFU/g, a pour-plate technique was applied. The plating media was *Listeria* Selective Agar Base (all reagents from Oxford Formulation, Oxoid, Hampshire, England). Plates were incubated for 48 h at 37°C. Plate counts for detection of *Leuc. carnosum* were made on BHI-agar plates, incubated for 5 d at 20°C.
Descriptive sensory profiling was made on control saveloys without *Leuc. carnosum* and samples inoculated with *Leuc. carnosum*. The sample variation was described with 10 descriptive terms. 5 or 6 assessors described the two samples after 1, 2, 3 and 4 weeks of incubation at 5°C.

Results and Discussion

Leuc. carnosum grew within 7 days to the maximum cell count of 10^8 CFU/g in the MA-packaged saveloys when an inoculum of 10^7 CFU/g was used. Three weeks were needed to reach the same cell count when an inoculum of 10^4 CFU/g was used.

L. monocytogenes grew in the MA-packaged saveloys from 10 CFU/g to between 10^5-10^6 CFU/g during the 4 weeks incubation period at 5 °C. Addition of *Leuc. carnosum* reduced the growth of *L. monocytogenes*. An even spreading of the protective culture was vital for the biocontrolling effect. Application of the protective culture by spraying with two nozzles during slicing reduced the growth of *L. monocytogenes* with approx. 10^4, however, some of the samples contained > 100 CFU/g *L. monocytogenes* after the 4 weeks storage period (Figure 1). The sliced surface of the saveloys was covered by the biocontrolling culture, but the culture was not distributed on the edge of the slices. Sprinkling of the surface of the peeled saveloys with the bioprotective culture before slicing reduced the level of *L. monocytogenes* to < 1 CFU/g in all samples after the 4 weeks storage period (Figure 1). An initial high number of the protective culture was necessary to prevent growth of *L. monocytogenes*. An inoculum of 10^4 CFU/g could not prevent growth of *L. monocytogenes* even when the bioprotective culture was both sprinkled on the surface of the saveloys and applied by nozzles during slicing (Figure 2).

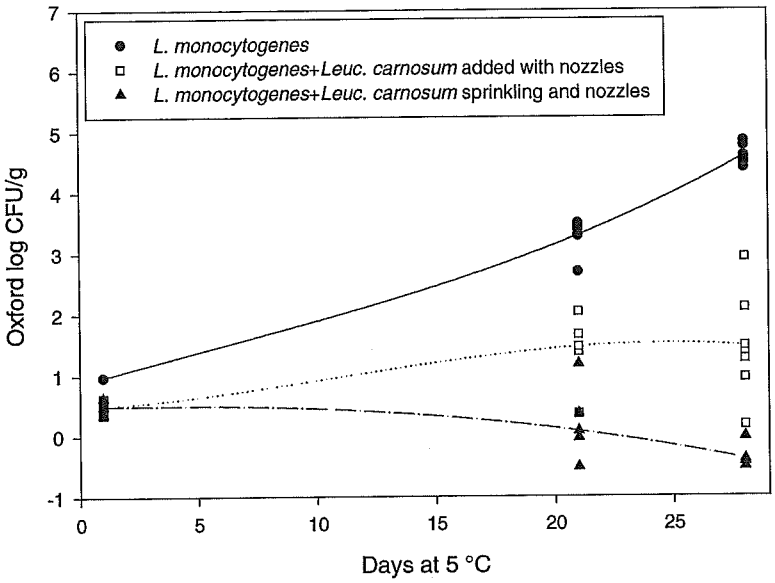

Figure 1. Growth of *Listeria monocytogenes* in MA-packaged saveloys with or without addition of 10^7 CFU/g *Leuconostoc carnosum*. (●) *L. monocytogenes* control; (□) *L. monocytogenes* with *Leuc. carnosum* applied during slicing by spraying with two nozzles; (▲)*L. monocytogenes* with *Leuc. carnosum* sprinkled onto the peeled surface of the saveloys in addition to spraying during slicing.

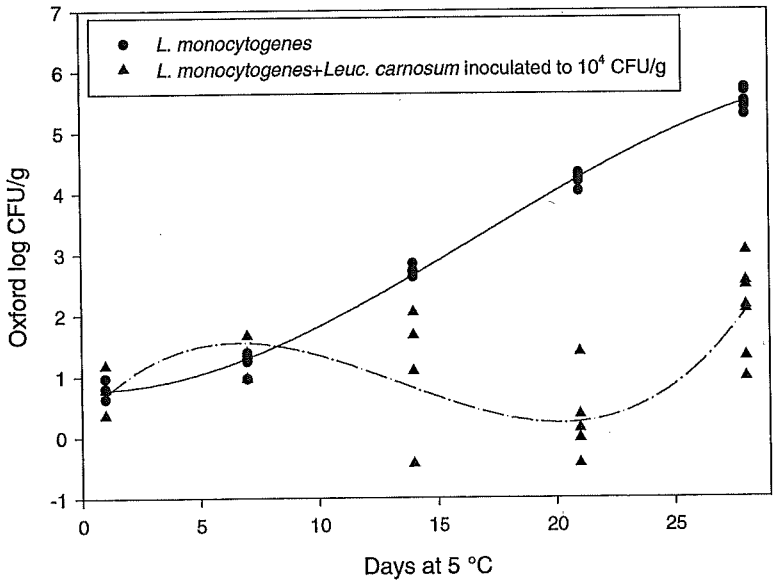

Figure 2. Growth of *Listeria monocytogenes* in MA-packaged saveloys with or without addition of 10^4 CFU/g *Leuconostoc carnosum* 4010. *Leuc. Carnosum* was sprinkled ono the surface of the peeled saveloys and applied during slicing by spraying with two nozzles. (●) *L. monocytogenes* control; (▲)*L. monocytogenes* and *Leuc. Carnosum.*

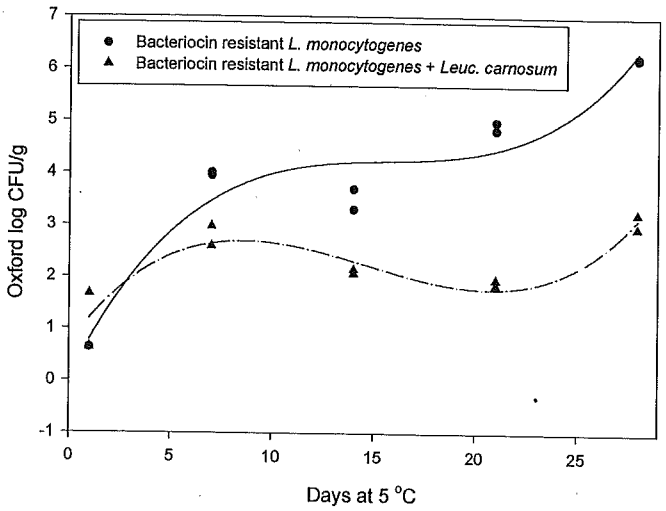

Figure 3. Growth of bacteriocin-resistant *Listeria monocytogenes* in MA-packaged saveloys with or without addition of 10^7 CFU/g *Leuconostoc carnosum*. (●) Bacteriocin-resistant *L. monocytogenes* control; (▲)Bacteriocin-resistant *L. monocytogenes* with *Leuc. carnosum*.

Resistance to class IIa bacteriocins occurs by one general mechanism in *L. monocytogenes* [2], and there is cross-resistance between the different members of this bacteriocin family. Almost 3% of approx. 400 *L. monocytogenes* strains were naturally resistant to the class IIa bacteriocin pediocin PA-1 [5]. However, growth of a bacteriocin-resistant strain of *L. monocytogenes* was markedly reduced by the biocontrolling culture (Figure 3). The result shows that the biocontrolling culture would still have a considerable effect even if a IIa-resistant listerial strain were to prevail in the product.

The descriptive sensory data were evaluated by principal component analysis (Unscrambler v7.5, CAMO, Norway) after normalization of the data of the 10 descriptors. There was not observed any grouping of the samples, except that the storage period influenced the freshness of the samples. Samples with or without *Leuc. carnosum* could not be separated. The results indicate that the sensory changes made by addition of *Leuc. carnosum* were smaller than the changes caused by the incubation period. Use of the biocontrolling culture will therefore not compromise the sensory quality of the tested product.

All in all, we seem to have overcome some of the major obstacles regarding the use of biocontrol for the preservation of certain meat products. This work has resulted in a temporary approval for the use of *Leuc. carnosum* DMRICC 4010 in Denmark, the first of its kind.

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